

Pavlin D, Goldman ES, Gluhak-Heinrich J, Magness M, *Zadro R. Orthodontically stressed periodontium of transgenic mouse as a model for studying mechanical response in bone: The effect on the number of osteoblasts. Clin Orthod Res 2000;3:55-66.

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The aim of this study was to biomechanically characterize a mouse tooth movement model and to evaluate the effect of orthodontic stress on the proliferation of periodontal osteoblasts. The authors used an orthodontic coil spring appliance with a low force/deflection rate, which produced an average force of 10-12 g. This design provided a predictable tipping movement of the molar with the center of rotation at the level of root apices. Histological observations of paradental tissues revealed a response favoring a fast onset of tooth movement and deposition of new osteoid starting after 3 days of treatment. The effect of treatment on the histomorphometric parameter of the number of osteoclasts per unit bone perimeter was determined after 1, 2, 3, 4, 6, and 12 days of treatment. Starting with day 2, the osteoblast number showed a modest but consistent increase in treated periodontal sites at all time-points, ranging from 14 to 39% and becoming significant only at day 6. Only a moderate increase in the number of osteoblasts in the areas of otherwise intense bone matrix synthesis suggests that, during bone formation, proliferation of cells has a smaller role compared to a marked increase in differentiation of individual cells. The mouse model, which allows for a controlled, reproducible, orthodontic mechanical loading, can be applied to both wild-type and transgenic animals and should enhance the research of the transduction of mechanical orthodontic signal into a biological response.

Mazul-Sunko B, Žarković N, Vrkić N, Klinger R, Perić M, Bekavac-Bešlin M, et al. Pro-atrial natriuretic peptide hormone from right atria is correlated with cardiac depression in septic patients. J Endocrinol Invest 2001;24:RC22-4.

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N-terminal pro-atrial natriuretic peptide [proANP(1-98)] has been extensively investigated in patients with chronic heart failure and ischemic heart disease. It is found to be a better marker of cardiac dysfunction than atrial natriuretic peptide (ANP). The possible involvement of proANP(1-98) in cardiac depression caused by sepsis has not been studied yet. Therefore, we analyzed atrial plasma concentration of proANP(1-98) in 17 septic patients with hemodynamic variables measured or calculated using pulmonary artery catheter. The results of altogether 96 measurements show a significant negative correlation of proANP(1-98) and cardiac index ($p < 0.024$), oxygen delivery ($p < 0.03$) and oxygen consumption ($p < 0.03$). There is also a positive correlation with pulmonary vascular resistance ($p < 0.03$). ProANP(1-98) is significantly higher in patients who developed acute respiratory distress syndrome (ARDS) ($p < 0.001$). This study implies that proANP(1-98) is a possible novel hormone marker of cardiac depression caused by sepsis that could be used for prediction of ARDS.

Bulić-Jakuš F, Strahinić-Belovari T, Marić S, Ježek D, Jurić-Lekić G, Vlahović M, et al. Chemically defined

protein-free in vitro culture of mammalian embryo does not restrict its developmental potential for differentiation of skin appendages. Cells Tissues Organs 2001;169:134-43.

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In a unique serum- and protein-free chemically defined in vitro culture model of postimplantation mammalian development the epidermis differentiates regularly, although the differentiation of other tissues is impaired due to the lack of the serum. The present study in that model was done to estimate more carefully the degree of epidermal differentiation in defined media supplemented with some growth- or differentiation-stimulating substances. The main objective was to discover by grafting in vivo to the richer environment whether simple protein-free culture conditions restrict an inherent embryonic potential for differentiation of skin appendages. Embryonic parts of E9.5 gastrulating Fischer rat embryos were cultivated for 2 weeks in the protein-free Eagle's minimum essential medium supplemented with holotransferrin, apotransferrin, insulin and/or Na_2SeO_3 and in controls cultivated in protein-free medium or in serum-supplemented medium. In all experiments there was a high incidence of differentiation of the epidermis. A high level of epidermal differentiation was confirmed for the first time at the ultrastructural level. A well-differentiated cornified layer and cells connected with desmosomes containing keratohyaline masses and cytokeratin filaments were found. A strong immunohistochemical signal for the proliferating cell nuclear antigen was always detected in the basal layer of the epidermis showing that those cells were still able to proliferate. Finally, embryos precultivated for 1 or 2 weeks in the protein-free medium and media supplemented with apotransferrin or serum were grafted under the kidney capsule for an additional 2 weeks. It was discovered that even after spending 2 weeks in the simple protein-free medium in vitro, embryos retained their developmental potential for differentiation of skin appendages (hair and sebaceous glands).

Puškar D, Balagović I, Filipović A, Knezović N, Kopjar M, Huis M, et al. Symptomatic physiologic hydronephrosis in pregnancy: incidence, complications and treatment. Eur Urol 2001;39:260-3.

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The authors analyzed 103 consecutive women who presented with clinical signs and symptoms related to the upper urinary system. Renal sonography, urinalysis, serum creatinine levels, white blood cell (WBC) count, and urine culture were done in all patients at first visit and repeated at least once a month until 1 month after delivery. In patients who manifested acute pyelonephritis, urinalysis, WBC count, erythrocyte sedimentation rate and C-reactive protein levels were repeated every 3 days until normalization, and urine culture as well as renal sonography were performed once a week until 1 month after delivery. Conservative measures (positioning, analgesia, antibiotics) were performed in all patients with symptomatic physiologic hydronephrosis. If the patient's condition was refractory to medical management, drainage of the ureter with a double pigtail stent was performed. Conservative measures were successful in 97 (94%) of 103 patients but 6 (6%) patients had ongoing signs and symptoms of acute pyelonephritis progressing to urosepsis. In all of them, antibiotics were continued and a double pigtail stent was placed resulting in fast regression of

symptoms, curing of renal infection and progress of the pregnancies to the term with vaginal delivery. Symptomatic hydro-nephrosis in pregnancy can be treated conservatively. If the patient's condition is refractory to medical management, an internal drainage with double pigtail stent may be necessary.

Vilović K, Sapunar D, Ilijić E, Mimica MD, England MA, Saraga-Babić M. Morphological characteristics of dying cells in axial structures of developing human embryos. *Cells Tissues Organs* 2001;169:347-54.

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The authors analyzed the morphological features of dying cells in the developing axial structures of 5 human embryos between 5 and 8 weeks of postovulatory age. Cell death in the axial structures, i.e. spinal cord, notochord and surrounding mesenchyme and somites, was analyzed using light and electron microscopy. Tissue samples were taken from the cervico-thoracic region of normal human conceptuses. Two morphological types of cell death were found: apoptosis which was characterized by round or semilunar nuclear chromatin condensations, condensation and shrinkage of the cytoplasm and formation of apoptotic bodies, and cell death without the morphological features of apoptosis which was characterized by pyknotic nuclear chromatin condensations, vacuolated cytoplasm and the formation of numerous intercellular spaces. Apoptotic death occurred during the 5th week of normal development in all the axial structures. Later, apoptotic death appeared in all the axial structures, with the exception of the notochord, where some dying cells displayed features of secondary necrosis. According to these findings, apoptosis seems to be the most frequently observed type of PCD, but it is not the exclusive type of morphological cell death during the development of axial structures in human embryos.

Momčilović B, Alkhatib HA, Duerre JA, Cooley M, Long WM, Harris TR, et al. Environmental lead-210 and bismuth-210 accrue selectively in the brain proteins in Alzheimer disease and brain lipids in Parkinson disease. *Alzheimer Dis Assoc Disord* 2001;15:106-15.

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The authors studied the occurrence of the environmental radon daughters, ²¹⁰Po (alpha particles), and ²¹⁰Bi (beta particles), in the protein and lipid fractions of cortical gray and subcortical white matter from the frontal and temporal lobes of human brains of persons with Alzheimer disease (AD), persons with Parkinson disease (PD), smokers, or persons with no previous evidence of clinical neurologic disease (controls). They found a 10-fold increase in ²¹⁰Po and ²¹⁰Pb radioactivity in the protein fraction from both the cortical gray and subcortical white matter in AD and smokers, and a similar increase in the lipid fraction in PD. The pathognomonic distribution of the radon daughters to the lipids in PD and to the proteins in AD was inferred to reflect the increase of local chlorine availability to which radon daughters bound selectively. Cigarette smoking strongly increases radon daughter retention in the central nervous system.

Lončarek K, Mustač E, Frković A, Prodan M. Prevalence of anencephaly in the region of Rijeka, Croatia. *Eur J Epidemiol* 2001;17:241-4.

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This retrospective study determines the prevalence of anencephaly in the region of Rijeka, Croatia. Records of all spontaneous and therapeutic abortions terminated in medical institutions, all fetuses weighing more than 500 g or more than 22 weeks gestation (whether the product of abortion, therapeutic termination, stillborn or liveborn) and infants who died in the

first year of life in the region of Rijeka, Croatia, during the 1963-2000 period were reviewed. There were 135,451 births; 22 of them were anencephalics (19 stillborn), which comprises 0.2% of all births and 2.1% of stillbirths. Annual prevalence of anencephaly varied in range from 0.00 to 7.42 per 10,000 births. In two cases pregnancy was electively terminated after ultrasonographic diagnosis of anencephaly. Fifteen anencephalics were female, six were male, and in one case sex was undetermined due to aplasia of genital organs. Associated congenital malformations were detected in 18 anencephalics. The importance of establishing national and international registers of congenital malformations in all countries is stressed. The authors suggested that the setting of obligatory reporting of all congenital malformations would be the first step toward this practice in Croatia, as well as in other developing countries.

Balog T, Marotti T, Abramić M, Beusan-Svoboda I, Sobočanec S, Hršak I. Neutrophil neutral endopeptidase variation and its regulation by opioid peptides. *Int Immunopharmacol* 2001;1:569-79.

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The opioid peptide methionine-enkephalin (MENK) has significant immunomodulatory ability in addition to its neurotransmitter function. Since neutral endopeptidase (NEP, CD10, enkephalinase EC 3.4.24.11) cleaves opioid peptides, the presence and activity of NEP in neutrophils from different persons might be responsible for the diverse, neuropeptide-induced, responses of neutrophils from different donors [Ann. N. Y. Acad. Sci. 650 (1992) 146]. The results obtained showed statistically significant differences in NEP activity among donors (high, medium and low). A 10-fold higher NEP activity in neutrophils (160-280 nmol/10⁶ cells/h) and in their corresponding membrane preparations (550 nmol/mg protein/min) in our study, as compared to literature data, was a result of high specificity and affinity of Suc-Ala-Ala-Phe-pNA as substrate. In control nontreated neutrophils, the number of CD10 positive cells were not correlated with NEP activity. However, in neutrophils treated with a physiological (10⁻¹⁰ M) concentration of MENK, two main events occurred; not only did the number of CD10 positive cells correlate with NEP activity, but contrary to control samples, MENK upregulated the expression of CD10 marker as demonstrated by an increase of mean fluorescence intensity (F-mean) in donors with low NEP activity. Taken together, these data add some clarity to the diverse activity of enkephalins in association with enzyme cleavage of those molecules.

***Bedenić B, Randegger CC, Stobberingh E, Hachler H. Molecular epidemiology of extended-spectrum beta-lactamases from *Klebsiella pneumoniae* strains isolated in Zagreb, Croatia. *Eur J Clin Microbiol Infect Dis* 2001;20:505-8.**

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In order to assess the molecular epidemiology of 40 previously identified extended-spectrum beta-lactamase (ESBL)-producing clinical isolates of *Klebsiella pneumoniae*, gene sequencing was performed. While the previous examination of these isolates revealed one TEM producer, the sequencing procedure performed in this study identified 13 additional TEM producers, and all of the sequenced genes reflected production of nonESBL TEM-1. All 38 suspected SHV producers were confirmed to be carriers of blaSHV-ESBL genes using the PCR/NheI test and sequencing. Among them, types SHV-2, SHV-5, and SHV-12 were found in 20, 10, and 7 isolates, respectively, and SHV-2a was identified in 1. SHV-5 and SHV-12 conferred higher resistance to ceftazidime and cefepime, while SHV-2 and SHV-2a raised the minimum inhibitory concentrations of cefotaxime and ceftipime. Fourth-generation cephalosporins were found to be more active against the isolates than third-generation cephalosporins.

***Fatović-Ferenčić S, Holubar K. Mal de Meleda: from legend to reality. *Dermatology* 2001;203:7-13.**

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Luca Stulli of Dubrovnik (Ragusa), 1772-1828, was one of the first to make epidemiologic studies of heritable skin disorders. His treatise of what became the 'mal de Meleda' on the Adriatic island of Mljet (Meleda) is a classic in the dermatologic literature. The present study documents his life, his birth record, his portrait and recalls his original publication..

Glojnaric I, Časl MT, Simić D, Lukač J. Serum amyloid A protein (SAA) in colorectal carcinoma. *Clin Chem Lab Med* 2001;39:129-33.

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The changes in serum levels of serum amyloid A protein were studied in 67 patients suffering from colorectal carcinoma and compared to three other major acute phase proteins: C-reactive protein, alpha₁-antichymotrypsin and alpha₁-acid glycoprotein. Although the presence of colorectal carcinoma caused an increase in serum levels of all the acute phase reactants studied, serum amyloid A protein showed the most powerful reaction in pre-operative disease stage, with the mean value of 330 mg/L (range 7-2506 mg/L) as compared to the normal values of <1.2 mg/L obtained in 30 healthy adults. The mean serum amyloid A protein concentration increased to 487 mg/L after surgery, declining during the post-operative clinical course until the sixth chemotherapy cycle (from 167 mg/L to 64 mg/L), but never returned to the normal range. In the later chemotherapy cycles, mean serum amyloid A protein increased to 163 mg/L, probably as a result of the disease relapse. According to the statistical relations among exact confidence intervals for proportions, serum amyloid A protein showed the best specificity for colorectal carcinoma of all the acute phase proteins studied (83-100%) and also a sensitivity of 100%. In conclusion, serum amyloid A protein seems to be a reliable parameter, which could be recommended for clinical routine as a non-specific tumour marker for colorectal carcinoma.

Pivac N, Muck-Šeler D, Barišić I, Jakovljević M, Puretić Z. Platelet serotonin concentration in dialysis patients with somatic symptoms of depression. *Life Sci* 200;68:2423-33.

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Platelet serotonin (5-HT) concentration was measured in 65 male and 45 female chronic renal patients on hemodialysis (HD) with different somatic symptoms of depression (crying spells, irritability, sleep disturbance, fatigability, loss of appetite, weight loss, somatic preoccupation and loss of libido), to find out the relationship between the severity of symptoms and platelet 5-HT concentration. Male and female patients had significantly lower platelet 5-HT concentration than 62 male and 38 female healthy subjects. Gender-differences in platelet 5-HT values observed in healthy subjects were not found in patients. Platelet 5-HT concentration differed in the groups of patients with the different scores of particular somatic symptoms (loss of appetite and loss of libido), but was similar in patients with other somatic symptoms. There was no relationship between platelet 5-HT concentration and the severity of somatic symptoms, or between platelet 5-HT concentration and age of the patients. Gender-related differences in the occurrence of somatic symptoms were detected in patients with the different degrees of weight loss, somatic preoccupation and loss of li-

bido. These results suggest that platelet 5-HT concentration could not be used as a biological marker for the severity of somatic symptoms in chronic renal patients on HD.

***Miličević Z, Profozić V, Wyatt J, Ristić S, Woodworth JR, Seger M, et al. Intramuscular injection of insulin lispro or soluble human insulin: pharmacokinetics and glucodynamics in Type 2 diabetes. *Diabet Med* 2001;18:562-6.**

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The aim of the study was to compare the pharmacokinetics and glucodynamics of insulin lispro and soluble human insulin following intramuscular (i.m.) injection in patients with Type 2 diabetes with secondary failure of sulphonylureas. Single 15-U i.m. doses of insulin lispro or soluble human insulin were administered to 16 patients in a two-way, randomized, crossover design. Glucodynamic and pharmacokinetic parameters were determined over 6 h after insulin injection using clamp techniques. Insulin C(max) was significantly higher (971 ± 217 vs. 659 ± 141 pmol/l, $p < 0.001$) and T(max) was significantly shorter (46.9 ± 27 vs. 94.7 ± 50.1 min, $p = 0.002$) with insulin lispro. Glucose infusion rate (GIR) curves showed clear separation 20 min after injection and were significantly greater for insulin lispro during the 40-60, 60-80 and 80-100-minute time intervals. Total glucose infused was only approximately 5% larger with insulin lispro during the 6-h follow-up, due to lower insulinaemia at later time points. The glucose R(max) and TR(max) were not statistically different between insulin treatments. This study shows that i.m. injection of insulin lispro is followed by its more rapid absorption, which results in stronger metabolic effect in the first 2 h when compared with soluble human insulin under the same test conditions.

***Sabolić I, Herak-Kramberger CM, Brown D. Subchronic cadmium treatment affects the abundance and arrangement of cytoskeletal proteins in rat renal proximal tubule cells. *Toxicology* 2001;165:205-16.**

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Disfunction of proximal tubules (PT) in cadmium (Cd) nephrotoxicity in mammals results from the diminished functional capacity of brush-border membrane (BBM) caused by (a) direct inhibition of BBM transporters by Cd, (b) shortening and loss of microvilli, and (c) loss of specific BBM transporters. In this work we studied the in vivo effect of CdCl₂-treatment in rats (2 mg Cd/kg b.m., s.c., daily for 14 days) upon abundance and arrangement of actin filaments, actin-bundling protein villin, and microtubules (MT) in PT cells. Cd-treatment elicited a dramatic accumulation of Cd in the kidney cortex (200 microg/g tissue wet mass after 14 days) and a strongly increased abundance of metallothionein in PT cells. As revealed by immunocytochemistry in tissue cryosections, the staining intensity of actin and villin in PT cells of Cd-treated rats was generally decreased, without a marked change in their intracellular distribution, whereas MT became largely irregular, diminished in most cells, and lost in many cells. However, the immunoblots revealed an increased content of villin and alpha-tubulin in cortical tissue homogenates from Cd-treated rats, thus indicating an impaired bundling of actin and greatly depolymerized MT in cells intoxicated with Cd. The partial loss of apical actin and villin in PT cells of Cd-treated rats may reflect (or cause) shortening and loss of microvilli, whereas derangement and depolymerization of MT may contribute to the impairment of intracellular recycling of BBM proteins, and lead to the loss of BBM transporters.